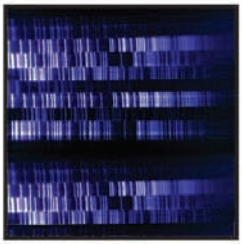


PCR clean-up
Gel extraction

User Manual

NucleoSpin® Extract II

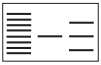
July 2009 / Rev. 09

MACHEREY-NAGEL

PCR clean-up, Gel extraction

Protocol-at-a-glance (Rev. 09)

NucleoSpin® Extract II

		PCR clean-up	Gel extraction
1	**PCR clean-up:** **Adjust binding condition** **Gel extraction:** **Excise DNA fragment /** **Solubilize gel slice**	200 µl NT / 100 µl PCR	 200 µl NT / 100 mg gel 50°C 5 - 10 min
2	**Bind DNA**	 11,000 x *g* 1 min	
3	**Wash silica membrane**	700 µl NT3 11,000 x *g* 1 min	
4	**Dry silica membrane**	11,000 x *g* 2 min	
5	**Elute DNA**	15 - 50 µl NE RT 1 min 11,000 x *g* 1 min	

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1 Components

1.1 Kit contents

	NucleoSpin® Extract II		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740609.10**	**740609.50**	**740609.250**
Binding Buffer NT	10 ml	2 x 25 ml	2 x 120 ml
Wash Buffer NT3 (Concentrate)*	6 ml	2 x 6 ml	40 ml
Elution Buffer NE**	5 ml	15 ml	50 ml
NucleoSpin® Extract II Columns (yellow rings)	10	50	250
Collection Tubes (2 ml)	10	50	250
User Manual	1	1	1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer NE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 - 100% ethanol

Consumables

- 1.5 ml microcentrifuge tubes
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes
- Heating block
- Vortex mixer
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this User Manual

It is strongly recommended reading the detailed protocol sections of this User Manual if the **NucleoSpin® Extract II** kit is used for the first time. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-a-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at *www.mn-net.com*.

2 Product description

2.1 The basic principle

With the **NucleoSpin® Extract II** method, DNA binds to a silica membrane in the presence of chaotropic salt added by Binding Buffer NT. The binding mixture is loaded directly onto **NucleoSpin® Extract II Columns**. Contaminations like salts and soluble macromolecular components are removed by a simple washing step with ethanolic Wash Buffer NT3. Pure DNA is finally eluted under low ionic strength conditions with slightly alkaline Elution Buffer NE (5 mM Tris/HCl, pH 8.5).

2.2 Kit specifications

- The **NucleoSpin® Extract II** kit is designed for the direct purification of PCR* products and for the purification of DNA from TAE/TBE agarose gels (two applications in one kit).
- The **NucleoSpin® Extract II** buffer formulation ensures complete removal of primers from PCR* reactions while small DNA fragments are still bound and purified with high recovery.
- With **NucleoSpin® Extract II** even DNA fragments from PCR* reaction buffers rich in various detergents can be purified with high recovery.
- The adsorption of DNA to the **NucleoSpin® Extract II** membrane is pH-dependent. Optimal recovery is achieved by using TAE standard gels or reaction mixtures with pH 6 - 8.
- Standard as well as low melting agarose gels can be used.
- The prepared DNA fragments can be used directly in applications like automated fluorescent DNA sequencing, PCR*, or any kind of enzymatic reaction.

* PCR is patented by Roche Diagnostics

Table 1: Kit specifications at a glance	
Parameter	**NucleoSpin® Extract II**
Elution volume	15 - 50 µl
Binding capacity	15 µg
Time/prep	10 min for 6 preps
Direct purification of amplified DNA	see section 5
Concentration, removal of salts, enzymes, nucleotides, and/or labeling reagents like biotin or radioactive ATP etc.	see section 5 and 7.1
DNA fragments from agarose gels	see section 6
Purification of reaction mixtures **without SDS**	see section 5
Purification of reaction mixtures **containing SDS**	see section 7.1
Purification of **single stranded DNA**	see section 7.2
Removal of small DNA fragments and **primer-dimers**	see section 2.3

2.3 Removal of small DNA fragments and primer-dimers

NucleoSpin® Extract II is designed to remove even traces of unused primers, and at the same time, to purify PCR products down to 65 bp. However, in some cases it is necessary to exclude these small fragments, e.g. primer-dimers or side products resulting from unspecific annealing, since they might interfere with your downstream sequencing or cloning applications.

Removal of double stranded DNA >65 bp can be achieved by diluting an aliquot of Buffer NT with sterile water in an appropriate ratio and then proceeding with the standard protocol (see section 5). Diluting Buffer NT in a certain range lowers the binding efficiency for small fragments without compromising the recovery of larger PCR products. Which dilution ratio to choose depends on the fragment size that is to be purified as well as on the PCR buffer system that is used.

Influence of fragment size: ***The smaller the fragment in question, the less you have to dilute Buffer NT.***

Influence of PCR buffer system: The influence of the PCR buffer system on the removal of small fragments is more complex. Some reaction buffers contain detergents

like Tween or high concentrations of additives like betaine to lower the melting temperature of the DNA template. These substances can usually be found in PCR buffers for high fidelity or long range PCR. They tend to lower the binding efficiency of DNA to the silica membrane and therefore have to be considered when choosing a dilution ratio of Buffer NT. ***As a rule of thumb if a PCR buffer system without special additives is used, adding 3 to 5 volumes of water to 1 volume of Buffer NT will lead to removal of small fragments up to 100 bp. Otherwise adding 1 to 3 volumes of water to 1 volume of Buffer NT will be sufficient.***

Therefore for each size of small fragments >65 bp that has to be removed, and for each PCR system, you can determine the appropriate ratio of Buffer NT dilution, in advance.

Figure 1 shows a purification result with a Buffer NT dilution series. Pure Buffer NT (lane 3), as well as Buffer NT plus one volume of water (lane 4), lead to 100% recovery of a PCR fragment ladder (lane 2). More diluted Buffer NT cuts off more and more of the low molecular mass bands. Usually a dilution with 5 volumes of water should be sufficient to eliminate even larger unwanted primer-dimer fragments while purifying the 164 bp fragment with >90%.

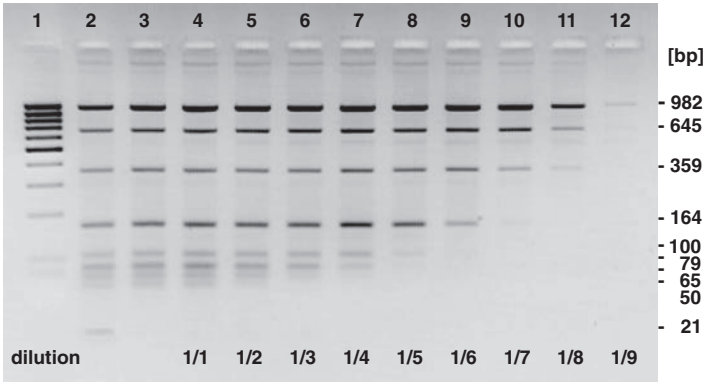


Figure 1: Purification of PCR reactions using Buffer NT dilutions

Lane 1: GeneRuler 100 bp DNA Ladder (MBI Fermentas)

Lane 2: DNA ladder input (21 base primer, 50, 65, 79, 100, 164, 359, 645 and 982 bp fragment) amplified using Biotaq DNA Polymerase (Bioline)

Lane 3: Purification with 100% Buffer NT

Lane 4-12: Purification with Buffer NT diluted with 1-9 volumes of water

* PCR is patented by Roche Diagnostics

2.4 Elution procedures

- For the elution of DNA, one of the following solutions can be used: Buffer NE (supplied, 5 mM Tris/HCl, pH 8.5), TE buffer, pH 8.5, distilled water, pH 8.5 or comparable low-salt buffer (important pH > 7).

- Note: EDTA in TE buffer may cause problems in subsequent reactions, and the pH of distilled water should be checked before use to avoid lower recovery. See Table 2 for the correlation between dispensed elution buffer volumes and typical recoveries for the purification of 1 - 5 µg of PCR* fragments (for gel extraction, recovery is approx. 10% lower).

- With an elution volume of 15 µl of Buffer NE, a typical recovery of 70 - 95% is usually obtained for DNA fragments between 50 - 10,000 bp resulting in highly concentrated eluates (see Table 2, Figure 2). If larger amounts (5 - 15 µg) of DNA have to be purified (e.g. from PCR* reactions > 100 µl or gel slices > 200 mg), elution with at least 50 µl of Buffer NE is recommended. Primers are not bound.

- Yields of larger fragments (> 5 - 10 kbp) can be increased by using preheated elution buffer (70°C): For elution, add preheated Elution Buffer NE and incubate for 1 - 2 min before collecting eluate by centrifugation. For fragments > 10 kbp the use of our **NucleoTraP®CR** kit is recommended.

Table 2: DNA recovery with NucleoSpin® Extract II

Fragment length	Elution volume	Recovery
65 bp	15 µl	85%
	25 µl	90%
	50 µl	95%
	100 µl	95%
400 bp	15 µl	85%
	25 µl	95%
	50 µl	100%
	100 µl	100%
700 bp	15 µl	85%
	25 µl	90%
	50 µl	95%
	100 µl	95%
1500 bp	15 µl	85%
	25 µl	85%
	50 µl	90%
	100 µl	95%

* PCR is patented by Roche Diagnostics

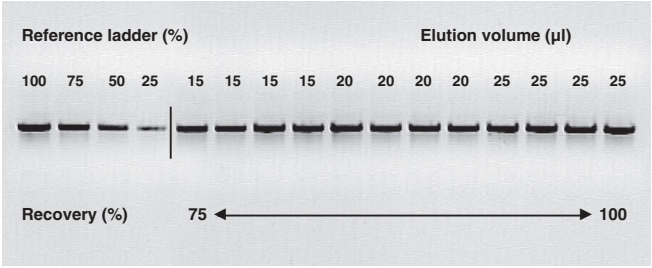


Figure 2: DNA recovery with different elution volumes

A PCR sample with a fragment size of 782 bp was purified from a 1% TAE agarose gel according to the standard protocol of **NucleoSpin® Extract II** using different elution volumes as shown. All elution volumes were adjusted to 25 µl plus 4.5 µl loading dye. For analysis the mixture was loaded on a 1% TAE agarose gel. The recovery was estimated by comparison with a fragment ladder.

3 Storage conditions and preparation of working solutions

Attention:

Buffer NT contains chaotropic salt. Wear gloves and goggles!

Storage conditions:

- The **NucleoSpin® Extract II** kit should be stored at room temperature and is stable for up to one year.

Before starting any **NucleoSpin® Extract II** protocol prepare the following:

- **Wash Buffer NT3:** Add the indicated volume of ethanol (96 - 100%) to **Buffer NT3 Concentrate**. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer NT3 at room temperature (20 - 25°C) for up to one year.

	NucleoSpin® Extract II		
	10 preps	**50 preps**	**250 preps**
Cat. No.	**740609.10**	**740609.50**	**740609.250**
Wash Buffer NT3 (Concentrate)	6 ml Add 24 ml ethanol	2 x 6 ml Add 24 ml ethanol to each bottle	40 ml Add 160 ml ethanol

4 Safety instructions – risk and safety phrases

The following components of the **NucleoSpin® Extract II** kits contain hazardous contents.

Wear gloves and goggles and follow the safety instructions given in this section.

Component	Hazard contents	Hazard symbol		Risk phrases	Safety phrases
NT	Guanidine thiocyanate	✖ Xn*	Harmful by inhalation, in contact with skin, and if swallowed	R 20/21/22	S 13

Risk phrases

R 20/21/22 Harmful by inhalation, in contact with skin, and if swallowed

Safety phrases

S 13 Keep away from food, drink, and animal feedstuffs

* Hazard labeling not necessary if quantity per bottle below 125 g or ml (certificate of exemption according to 67/548/EEC Art. 25, 1999/45/EC Art. 12 and German GefStoffV § 20 (3) and TRGS 200 7.1). For further information see Material Safety Data Sheet.

5 Protocol for PCR clean-up

The following protocol is suitable for PCR clean-up as well as concentration and removal of salts, enzymes, etc. from samples without SDS.

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Adjust DNA binding condition

Mix **1 volume of sample** with **2 volumes** of **Buffer NT** (e.g. mix 100 µl PCR reaction and 200 µl Buffer NT).

+ 2 vol NT per 1 vol sample

For sample volumes < 100 µl adjust the volume of the reaction mix to 100 µl using Buffer NT or water.

For removal of DNA fragments > 65 bp, dilutions of Buffer NT can be used instead of 100% Buffer NT. Please refer to section 2.3.

2 Bind DNA

Place a **NucleoSpin® Extract II Column** into a Collection Tube (2 ml) and load the sample.

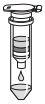
Load sample

Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through and place the NucleoSpin® Extract II Column back into the Collection Tube.

11,000 x *g* 1 min

3 Wash silica membrane

Add **700 µl Buffer NT3** to the NucleoSpin® Extract II Column. Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through and place the NucleoSpin® Extract II Column back into the Collection Tube.



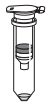
+ 700 µl NT3

Note: Carry-over of chaotropic salt may appear in suboptimal A_{230} values. To prevent problems due to chaotropic salt carry-over e.g. in very sensitive downstream applications or if the entire eluate has to be used, follow the instructions given in section 8.1 ("Suboptimal performance of DNA in sequencing, restriction, or ligation reactions - Carry-over of chaotropic salts").

11,000 x *g* 1 min

4 Dry silica membrane

Centrifuge for **2 min** at **11,000 x *g*** to remove **Buffer NT3** quantitatively. Make sure the spin column does not come in contact with the flow-through while removing it from the centrifuge and the Collection Tube.



Residual ethanol from Buffer NT3 might inhibit subsequent reactions and has to be removed in this step. In addition to centrifugation, total removal can be achieved by incubation of NucleoSpin® Extract II Columns for 2-5 min at 70°C prior to elution.

11,000 x *g*
2 min

5 Elute DNA

Place the NucleoSpin® Extract II Column into a **clean** 1.5 ml microcentrifuge tube (not provided). Add **15-50 µl Buffer NE** and incubate at **room temperature** for **1 min** to increase the yield of eluted DNA. Centrifuge for **1 min** at **11,000 x *g***.

+ 15-50 µl NE

Yield of larger fragments (>5-10 kbp) can be increased by using prewarmed elution buffer (70°C): For elution, add prewarmed elution buffer and incubate at room temperature for 1 min before collecting eluate by centrifugation.

11,000 x *g*
1 min

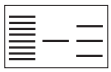
6 Protocol for DNA extraction from agarose gels

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Excise DNA fragment / Solubilize gel slice

Take a clean scalpel to excise the DNA fragment from an agarose gel. Excise gel slice containing the fragment carefully to minimize the gel volume. Determine the weight of the gel slice and transfer it to a clean tube.



For each **100 mg of agarose gel** add **200 µl Buffer NT**.

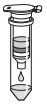
For gels containing >2% agarose, double the volume of Buffer NT. The maximum amount of gel slice per NucleoSpin® Extract II Column is 400 mg or 200 mg of a high percentage gel >2%. In this case 2 loading steps are required (step 2).

+ 200 µl NT per 100 mg gel

Incubate sample for **5 - 10 min** at **50°C**. Vortex the sample briefly every 2 - 3 min until the gel slice is **completely** dissolved!

50°C 5 - 10 min

2 Bind DNA



Load sample

Place a **NucleoSpin® Extract II Column** into a Collection Tube (2 ml) and load the sample.

Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through and place the NucleoSpin® Extract II Column back into the Collection Tube.

11,000 x *g* 1 min

3 Wash silica membrane

Add **700 µl Buffer NT3** to the NucleoSpin® Extract II Column. Centrifuge for **1 min** at **11,000 x *g***. Discard flow-through and place the NucleoSpin® Extract II Column back into the Collection Tube.

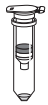
+ 700 µl NT3

Note: Carry-over of chaotropic salt may appear in suboptimal A_{230} values. To prevent problems due to chaotropic salt carry-over e.g. in very sensitive downstream applications or if the entire eluate has to be used, follow the instructions given in section 8.1 ("Suboptimal performance of DNA in sequencing, restriction, or ligation reactions - Carry-over of chaotropic salts").

11,000 x *g* 1 min

4 Dry silica membrane

Centrifuge for **2 min** at **11,000 x *g*** to remove **Buffer NT3** quantitatively. Make sure the spin column does not come in contact with the flow-through while removing it from the centrifuge and the Collection Tube.



Residual ethanol from Buffer NT3 might inhibit subsequent reactions and has to be removed in this step. In addition to centrifugation, total removal can be achieved by incubation of NucleoSpin® Extract II Columns for 2-5 min at 70°C prior to elution.

11,000 x *g*
2 min

5 Elute DNA

Place the NucleoSpin® Extract II Column into a **clean** 1.5 ml microcentrifuge tube (not provided). Add **15-50 µl Buffer NE** and incubate at **room temperature** for **1 min** to increase the yield of eluted DNA. Centrifuge for **1 min** at **11,000 x *g***.

+ 15-50 µl NE

Yield of larger fragments (>5-10 kbp) can be increased by using prewarmed elution buffer (70°C): For elution, add prewarmed elution buffer and incubate at room temperature for 1 min before collecting eluate by centrifugation.

11,000 x *g*
1 min

7 Support protocols

7.1 Concentration and removal of salts, enzymes, etc. Purification of samples containing SDS (Buffer NTB)

The NucleoSpin® Extract II Buffer NT is compatible with most commonly used detergents except sodium dodecyl sulfate (SDS). For purification of DNA from samples without SDS the standard protocol for PCR clean-up can be used (see section 5). For purification of DNA from SDS containing buffers, e.g. in applications like “Chromatin Immunoprecipitation” (CHiP), the SDS compatible Binding Buffer NTB can be used.

Note: Buffer NTB has to be ordered separately (150 ml Buffer NTB, Cat. No. 740595.150, see ordering information).

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Adjust DNA binding condition

Mix **1 volume of sample** with **5 volumes** of **Buffer NTB** (e.g. 100 µl reaction mix with 500 µl Buffer NTB).

+ 5 vol NTB per 1 vol sample

Note: If SDS starts to precipitate add 1 volume of isopropanol.

2 Bind DNA

Continue with **step 2** of the protocol for direct purification of PCR products (section 5).

7.2 Purification of single stranded DNA (Buffer NTC)

The NucleoSpin® Extract II Buffer NT is able to bind single stranded DNA (ssDNA) >150 bases. Shorter oligonucleotides, especially primers, are completely removed. If you need to purify short ssDNA the additional Binding Buffer NTC can be used (see Figure 3).

Note: Buffer NTC has to be ordered separately (100 ml Buffer NTC, Cat. No. 740654.100, see ordering information).

Before starting the preparation:

- Check if Wash Buffer NT3 was prepared according to section 3.

1 Adjust DNA binding condition

Mix **1 volume of sample** with **2 volumes** of **Buffer NTC** (e.g. 100 µl PCR reaction mix and 200 µl Buffer NTC).

If your sample contains large amounts of detergents or other critical substances, double the volume of Buffer NTC.

+ 2 vol NTC per 1 vol sample

2 Bind DNA

Continue with **step 2** of the protocol for direct purification of PCR products (section 5).

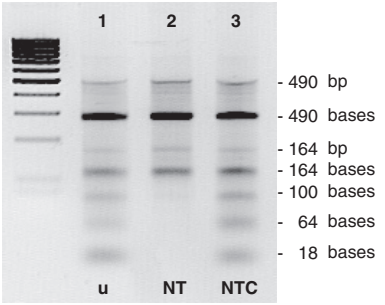


Figure 3 Purification of dsDNA and ssDNA using buffers NT and NTC

PCR fragments, amplified using one phosphorylated and one dephosphorylated primer, were partially digested with λ-Exonuclease. Samples were purified using Binding Buffer NT and NTC and run on a 1% TAE agarose gel. Remaining double stranded DNA can be seen as faint bands. The corresponding single stranded DNA is running slightly faster due to secondary structure formation. Compared to the input DNA (u, lane 1), Buffer NT removes ssDNA <150 bases (NT, lane 2), whereas Buffer NTC leads to full recovery of even primer oligonucleotides (NTC, lane 3).

8 Appendix

8.1 Troubleshooting

Problem	Possible cause and suggestions
Incomplete lysis of agarose slices	*High amount of agarose* • Use doubled volumes of Buffer NT for highly concentrated and/or LMP (low melting point) agarose gels. *Time and temperature* • Check incubation temperature. Depending on the weight of the gel slice, incubation (section 6, step 1) can be prolonged up to 20 min. Vortex every 2 min and check integrity of the gel slice. Very large gel slices can be crushed before addition of Buffer NT.
Low DNA yield	*Reagents not prepared properly* • Add indicated volume of 96 - 100% ethanol to Buffer NT3 Concentrate and mix well before use. *Incompletely dissolved gel slice* • Increase time or add another two volumes of Buffer NT and vortex the tube every 2 minutes during incubation at 50°C. Small pieces of gel are hardly visible and contain DNA that will be lost for purification. *Insufficient drying of the **NucleoSpin® Extract II** silica membrane* • Centrifuge 5 min at 11,000 x *g* or incubate column for 2 - 5 min at 70°C before elution to remove ethanolic Buffer NT3 completely. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). Remove the spin cup carefully from centrifuge and collection tube and avoid contact of spin cup with flow-through. *Not enough elution buffer* • Especially when larger amounts of DNA (> 5 µg) are bound, increase elution buffer volume up to 100 µl. *Isolation of large DNA fragments* • Preheat Elution Buffer NE to 70°C, and incubate on the silica membrane at room temperature for 2 min before centrifugation.

Problem	Possible cause and suggestions
Appearance of additional bands on agarose gel	*Appearance of additional bands on agarose gel* • In case water is used for elution and agarose with a low ion content is used for agarose gel electrophoresis, the formation of denaturated (single-stranded) DNA might be promoted. To re-anneal the DNA, add all components of the subsequent enzymatic reaction omitting the enzyme. Incubate at 95°C for 2 min and let the mixture cool slowly to room temperature (at this step the DNA re-anneals). Add the enzyme and continue with your downstream application.
Suboptimal performance of DNA in sequencing, restriction, or ligation reactions	*Carry-over of ethanol/ethanolic Buffer NT3* • Centrifuge 5 min at 11,000 x *g* or better incubate column for 5 - 10 min at 70°C before elution to remove ethanolic Buffer NT3 completely. Ethanolic contaminations are also indicated by gel-loading problems (samples float out of gel slots). Remove the spin cup carefully from the centrifuge and collection tube, and avoid contacting the spin cup with the flow-through. *Carry-over of chaotropic salts* • Modify washing and/or drying step in case of of sensitive downstream applications to remove last traces of Buffer NT (Note: The volume of Buffer NT3 included in the kit might not be sufficient when performing these modifications. Buffer NT3 can be ordered separately, see ordering information.): 1) Add 250 µl Buffer NT3 to the NucleoSpin® Extract II Column at the drying step (section 5 or 6, step 4). 2) Perform a second washing step with 700 µl Buffer NT3 (section 5 or 6, step 3) and add 250 µl Buffer NT3 at the drying step (section 5 or 6, step 4). *Elution of DNA with buffers other than Buffer NE, e.g. TE buffer (Tris/EDTA)* • EDTA might inhibit sequencing reactions. In this case it is recommended to re-purify DNA and elute in Buffer NE or water. *Not enough DNA used for sequencing reaction* • Quantify DNA by agarose gel electrophoresis before setting up sequencing reactions.

Problem	Possible cause and suggestions
Suboptimal performance of DNA in NanoDrop® Spectrophotometer Analysis or Agilent's Bioanalyzer	*Carry-over of traces of silica particles* • NanoDrop® Spectrophotometer technology is very sensitive to any particles included in the sample material. To pellet the silica particles centrifuge >2 min at 11,000 x *g* and take the supernatant for further use.
Low ratio A_{260}/A_{230}	*Carry-over of chaotropic salts* • Refer to detailed troubleshooting "Suboptimal performance of DNA in sequencing, restriction, or ligation reactions - Carry-over of chaotropic salts".

8.2 Ordering information

Product	Cat. No.	Pack of
NucleoSpin® Extract II	740609.10/.50/.250	10/50/250
Buffer NT	740614.100	100 ml
Buffer NTB	740595.150	150 ml
Buffer NTC	740654.100	100 ml
Buffer NT3 Concentrate (for 100 ml Buffer NT3)	740598	20 ml
Collection Tubes (2 ml)	740600	1000

Visit *www.mn-net.com* for more detailed product information.

8.3 References

Vogelstein B., and D. Gillespie. 1979. Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA 76: 615-619.

8.4 Product use restriction / warranty

NucleoSpin® Extract II kit components were developed, designed, distributed, and sold **FOR RESEARCH PURPOSES ONLY.** They are suitable **FOR *IN-VITRO* USES ONLY**. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is rather the responsibility of the user to verify the use of the **NucleoSpin® Extract II** kit for a specific application range as the performance characteristic of this kit has not been verified to a specific organism.

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